Acta vet. scand. 1965, *6*, 208—216.

From the Department of Obstetrics and Gynaecology, The Royal Veterinary and Agricultural College, Copenhagen, Denmark.

THE PATHOGENESIS OF HYDRALLANTOIS BOVIS

II. ELECTRICAL POTENTIAL AND CHEMICAL GRADIENTS BETWEEN THE ALLANTOIC FLUID AND THE MATERNAL BLOOD IN HYDRALLANTOIS AND NORMAL PREGNANCY*)

By

J. M. Skydsgaard

In cases of hydrallantois the allantoic fluid differs from normal allantoic fluid in the corresponding period of the pregnancy (*Skydsgaard* 1965). The difference may be due to foetal polyuria resulting in large amounts of fluid, relatively low creatinine concentrations and sodium, potassium and chloride concentrations which are close to those of extracellular fluid.

As discussed in the work referred to, the cause may also be due to changes in ion transport across the chorio-allantoic membranes. *Crawford & McCance* (1960) have shown an active sodium transport across in vitro preparations of swine chorion. The transport took place towards the maternal side, and in agreement with this an electrical potential difference was found across the isolated tissue with the maternal side positive. Electrical potentials of the same sign have also been demonstrated between the allantoic fluid and the mother in normal rabbits (*Wright* 1963; *Krespie & Davies* 1963), in goats (*Meschia et al.* 1958) and in sheep and cats (*Widdas* 1961).

An active transport of ions across the chorio-allantoic membranes thus seems to be an important part of the regulation of the foetal fluids. Furthermore these investigations showed that the chorio-allantoic membranes have an electrical resistance sufficient to maintain a relatively high electrical potential across

*) This investigation has been supported by Statens almindelige Videnskabsfond and Novofonden.

the membranes. Either changes of active ion transport or increase of permeability across the chorio-allantoic membranes could result in the fluid disturbance seen in hydrallantois.

In an attempt to further understand the role of these two factors in vivo measurements of the electrical potential gradient and the chemical gradients of sodium, chloride and potassium were made across the chorio-allantoic membrane. The permeability of this tissue was studied in vitro.

MATERIAL AND METHODS

The electrical potential and the chemical gradients of sodium, potassium and chloride were examined in vivo in five normal cows (Jersey) and eight cows suffering from excessive hydrallantois (Red Danish and Jersey) without primary foetal deformity and without hydramnion. The permeability of the chorio-allantoic membrane to sodium, potassium, chloride and creatinine was studied in tissues from six of the animals.

The electrical potentials and the chemical gradients in vivo.

Assuming equilibrium in a system consisting of two fluids separated by a semipermeable membrane, an ion which is passively transported across the membrane is distributed according to Nernst's equation (see *Ussing* 1960):

$$E = \frac{RT}{zF} \ln \frac{(C_1)}{(C_2)}, \text{ in which}$$

E is the electrical potential (mV) across the membrane, R the gas-constant, T the absolute temperature (C°), z the charge of the ion, F Faradays number, and (C_1) and (C_2) are the concentrations of the ion in the two fluids.

With regard to the chorio-allantoic membranes at the body temperature of the cow the distribution of a monovalent ion subject to passive transport alone would be described by the expression

$$E = \pm 62 \log \frac{(C_1)}{(C_2)}$$

Deviations from this relationship would indicate that the substance is not in equilibrium between the two compartments.

To examine whether the distribution of sodium, potassium and chloride agreed with the electrical potentials the following procedure was carried out.

Immediately following the collection of samples of the maternal blood and the allantoic fluid the electrical potential between these was measured. By laparotomy and hysterotomy a small area of the paraplacental chorio-allantois was uncovered. A sample of allantoic fluid was collected through a small needle. On removal of the needle a bridge consisting of saturated potassium chloride with 3 % agar in

a polyethylene tube was inserted through the opening and fixed with a ligature. Another bridge was inserted in a maternal vein (v. jugul. or v. mamm. ext.) immediately after the removal of the blood sample. The bridges were connected with a potentiometer (Model 27, Radiometer, København) through calomel half cells, and the potential was recorded. The analyses were carried out as mentioned in the previous paper.

The permeability of chorio-allantois in vitro was examined establishing a concentration gradient across the tissue and measuring the concentration change during the experimental period.

Samples of the paraplacental chorio-allantois were surgically collected, stored in oxygen saturated Ringer solution, and within an hour the tissue was placed between two acryl chambers. The chorionic surface of the tissue was bathed with Ringer solution, and the allantoic surface with the same solution diluted 1:5 with isotonic glucose. An isotonic creatinine solution was added to obtain creatinine concentrations of about 1500 µg/ml on the allantoic side. The tissue, chambers and their attachments were essentially the same as that described by *Ussing & Zerahn* (1951) and were kept at 39°C in a thermoregulated room. The storage and bathing solution used was Krebs-Ringer-HCO_3-glucose solution (pH 7.4) gassed with 95 % O_2 and 5 % CO_2 (*Cohen* 1951).

The electrical potential was measured with the same Ringer solution on both sides, and it was found to be zero when paraplacental tissue was used. Forty-five ml of fluid were added on both sides of the membrane. The system was allowed to equilibrate in 30 minutes and samples were then removed every 45 minutes.

Oedema in the membranes is a common feature in hydrallantois and although the oedema apparently disappeared within ½—1 hour of storage in the collecting solution it could be a considerable source of error. The results are given as concentration gain in the chamber containing the lower concentration of the substance to provide a measure of relative permeability, since experimental conditions did not allow calculation of permeability constants.

RESULTS

Table 1 shows the measured electrical potential gradients and the calculated chemical potential gradients between the allantoic fluid and the maternal blood. In the control animals the maternal blood was constantly electropositive in proportion to the allantoic fluid. The potential gradients ranged from 30 to 70 mV. The relationship between the electrical potential gradient and the chemical potential gradients suggested that the distribution of chloride closely approached its electrochemical equilibrium, but that the concentration gradient of *sodium* was maintained against the electrical potential. The chemical potential of *potassium* appears to vary independently of the electrical potential both as to sign and magnitude.

Table 1. Electrical (E) and chemical potentials (CP) between allantoic fluid and maternal blood in normal cows and cows with hydrallantois.

Condition	Foetal age in months	Potential gradients between allantoic fluid and maternal blood. The charge refers to maternal side			
		E (mV)	CP × — 1 (mV)		
			Cl^-	Na^+	K^+
Normal	8	+ 71	+ 71	— 28	— ∞
„	7	+ 66	+ 75	— 34	— 72
„	8	+ 64	+ 72	— 37	—106
„	4½	+ 45	+ 54	— 45	+ 41
„	6½	+ 28	+ 34	— 34	+ 53
Hydrallantois (120 l)	8	+ 11	+ 7.9	— 3.8	+ 22
Hydrallantois (90 l)	8	+ 9.6	+ 4.3	— 7.5	+ 31
Hydrallantois (90 l)	8	+ 9.0	+ 9.6	— 5.6	— 9.1
Hydrallantois (150 l)	6	+ 6.0	+ 2.0	— 0.6	+ 9.3
Hydrallantois (170 l)	8½	— 15	+ 2.8	— 0.8	— 6.0
Hydrallantois (140 l)	8	— 16	+ 1.6	— 4.7	+ 35
Hydrallantois (130 l)	7	— 30	+ 3.1	— 5.4	+ 25

In four cases of hydrallantois the maternal blood was electropositive and the distribution of the ions was similar to that of normal cows, but both electrical and chemical potentials were much smaller than those of normal pregnancy.

In three cases of hydrallantois the maternal blood was electronegative to the allantoic fluid. The chemical potentials for sodium and chloride were very small but of the same sign as those of normal animals.

These results as a whole showed the closest relation between the chemical potential gradient of chloride and the electrical potential gradient. This relationship in normal as well as in sick animals demonstrated that chloride was close to electrochemical equilibrium until the chloride concentration of the allantoic fluid reached about 90 mEq/l (Fig. 1). At concentrations above 90 mEq/l this no longer held true.

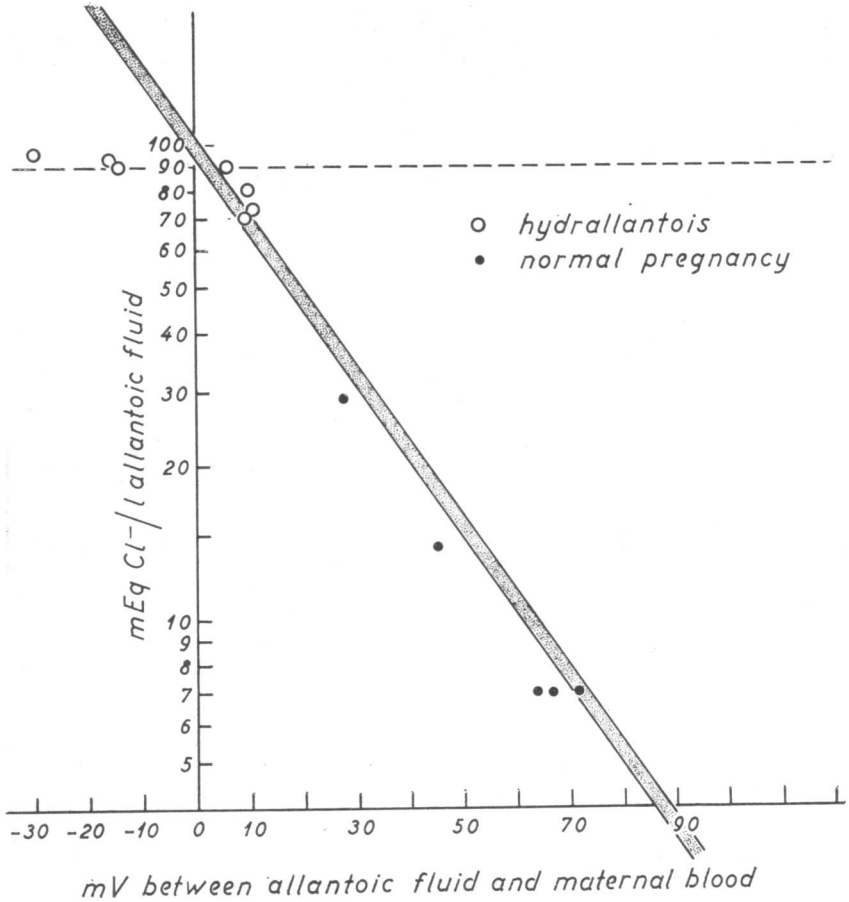

Figure 1. The figure shows relations between Cl^- concentrations in the allantoic fluid (logarithmic ordinate), and the electrical potential between allantoic fluid and the maternal blood (sign refers to mother, abscisse). The hatched area shows the theoretical electrochemical equilibrium for Cl^- between the allantoic fluid and maternal blood (plasma concentrations 95—105 mEq/l Cl^-).

As shown in Table 2, the permeability of the chorio-allantoic membranes appeared to be very pronounced in the cases of hydrallantois with electronegative maternal blood as compared to the animals with electropositive maternal blood.

It should be pointed out that the patients in which the maternal blood was found electronegative in relation to the allantoic fluid were all in a very poor condition at the arrival to the clinic, and the uterine distension was extreme in relation to the size of the animal.

T a b l e 2. Relationship of chorio-allantoic permeability to the chloride concentration in the allantoic fluid, and the electrical potential (E) between maternal blood and allantoic fluid.

Condition	In vivo measurements		In vitro measurements. Gain in concentration during $1^1/_2$ hour period			
	Cl^-, mEq/l allantoic fluid	E* mV	allantoic side Na mEq/l	K mEq/l	Cl mEq/l	chorionic side creatinine µg/ml
Normal	7	+ 71	0	0.0	0	6.3
Hydrallantois	74	+ 11	3	0.1	5	8.5
„	81	+ 10	0	— 0.1	0	—
„	90	+ 7	1	0.2	0	5.7
„	90	— 15	13	0.5	10	45
„	95	— 30	22	1.0	21	202

* Sign refers to maternal blood.

DISCUSSION

The origin of the electrical potential is not known in the cow, but could be explained, at least in part, by active sodium transport into maternal blood which appears to be a common feature among mammals (*Widdas* 1961). This suggestion is supported by the findings that the bovine maternal blood was electropositive to the allantoic fluid and that the concentration gradient of sodium was maintained against the electrical potential.

In cases of hydrallantois in which the maternal blood was electropositive, the electrical potential and the chemical gradients were much smaller, but the pattern was still the same, chloride was distributed according to its equilibrium, the concentration gradient of sodium was maintained against the electrical potential, and the distribution of potassium was apparently independent of any measured feature. Thus these cases support the hypothesis that hydrallantois includes either a decreased active sodium transport with decreased electrical potentials or an alteration of the placental structure with increased permeability (*Skydsgaard* 1965).

In some cases of hydrallantois the permeability indicates an altered structure of the chorio-allantoic membranes. However, this complication was in the few cases examined only significant simultaneous with chloride concentrations of 90 mEq/l or above in the allantoic fluid and with the maternal blood electronegative to the allantoic fluid. Since these were also very sick animals in

late stages of hydrallantois, it is tempting to regard the increased permeability as a late secondary complication. If the exponential increase of the amount of allantoic fluid in normal animals (*Skydsgaard* 1965) is an expression of a balance between its arrival and absorption and dependent upon active reabsorption of sodium, a decreased active sodium transport could cause a considerable increase in the rate of allantoic fluid accumulation. This might well cause a structural alteration due to acute distension in the late course of the disease.

Increased permeability and neutralization of concentration gradients across the membranes would be expected to result in an electrical potential approaching zero. It is therefore difficult to explain the reversed electrical potentials of up to 30 mV measured in those cases with allantoic fluid chloride concentrations of 90 mEq/l and above. Considering the associated large increases in the permeability of these tissues it is tempting to consider the potentials as spurious due to mechanical and irreversible destruction of the chorio-allantoic membrane.

It is interesting that the chemical potential of potassium across the membrane complex seems to vary independently of the electrical potential both as to its magnitude and sign. As the concentrations of potassium in normal allantoic fluid are found to vary from about 0.1 mEq/l to about 80 mEq/l, the membrane complex between the bovine allantoic fluid and the maternal blood seems to be rather impermeable to potassium. This suggests that the exchange of potassium between the bovine foetus and the mother might be limited to the intraepithelial capillaries found in chorion (*Björkman & Bloom* 1957).

CONCLUSIONS

I. Electrical potentials of approximately 30 to 70 mV were measured between the allantoic fluid and maternal blood (maternal blood positive) in normal pregnant cows. The chemical gradient of chloride across the tissue indicated its passive transport while the distribution of sodium appeared to be maintained against its electrochemical gradient suggesting active transport of sodium from allantoic fluid to maternal blood. The variable relationship between the chemical potential of potassium and electrical gradient indicated the tissues may be relatively impermeable to this ion.

II. In cases of hydrallantois bovis without primary congenital deformity very low electrical potentials and concentration gradients of ions were found between the allantoic fluid and the maternal blood. In cases in which the allantoic fluid chloride concentrations were less than 90 mEq/l the electric potentials were smaller but of the same sign as those of normal pregnancy. In cases where the allantoic fluid chloride concentrations were above 90 mEq/l the electrical potential changed sign and the permeability of chorio-allantois was distinctly increased.

III. The pathogenesis of hydrallantois bovis without primary congenital deformity seems to be connected with a decreased active sodium transport across chorion. A destruction of the micro-structure and altered permeability of the chorio-allantoic membrane in some cases might be a late secondary factor in the disease.

ACKNOWLEDGMENT

I wish to thank Dr. C. E. Stevens, N.Y.S. Veterinary College, Cornell University, for his advice and help during this work, and for valuable surgical assistance I want to thank Dr. L. H. Hansen, Dr. E. Stougaard, and Dr. J. Schou.

REFERENCES

Björkman, N. & G. Bloom: On the fine structure of the foetal-maternal junction in the bovine placentome. Z. Zellforsch. 1957, *45*, 649—659.

Cohen, P. P.: Preparation of Krebs-Ringer-Phosphate and bicarbonate solutions. In "Manometric Techniques and Tissue Metabolism", page 119. Ed. Umbreit et al., Minneapolis 1951.

Crawford, J. D. & R. A. McCance: Sodium transport by the chorio-allantoic membrane of the pig. J. Physiol. (Lond.) 1960, *151*, 458—471.

Krespie, V. & J. Davies: Electrical potential differences across the foetal membranes of the rabbit. J. Embryol. exp. Morph. 1963, *11*, 167—174.

Meschia, G., A. S. Wolkoff & D. H. Barron: Difference in electric potential across the placenta of goats. Proc. Nat. Acad. Sci. (Wash.) 1958, *44*, 483—484.

Skydsgaard, J.: The pathogenesis of hydrallantois bovis. I. The concentrations of sodium, potassium, chloride and creatinine in the foetal fluids in cases of hydrallantois and during normal pregnancy. Acta vet. scand. 1965, *6*, 193—207.

Ussing, H. H.: The alkali metal ions in isolated systems and tissues. (From 30th vol. of "Handbuch der experimentellen Pharmakologie" edited by Eichler & Farah). Berlin 1960, pg. 45—143.

Ussing, H. H. & K. Zerahn: Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. Acta physiol. scand. 1951, *23*, 110—127.

Widdas, W. F.: Transport mechanisms in the foetus. Brit. med. Bull. 1961, *17*, 107—111.

Wright, G. H.: Differences of electrical potential between maternal and foetal fluid compartments in the rabbit. J. Physiol. (Lond.) 1963, *166*, 20—21.

SUMMARY

Compared to normal pregnancy an evident reduction of the electrical potentials and electrochemical gradients was found in cases of hydrallantois without primary foetal deformity. The permeability of chorio-allantois in vitro was distinctly increased in some extreme cases of the disease.

It is suggested that the pathogenesis may be a primary decrease in active transport of ions across the chorion, followed by a mechanical destruction of the micro-structure of the chorio-allantoic membranes.

ZUSAMMENFASSUNG

Die Pathogenese der Hydrallantois bovis.

II. Elektrische Potentiale und chemische Gradienten zwischen Allantoisflüssigkeit und Mutterblut beim Vorliegen der Hydrallantois im Gegensatz zur normalen Trächtigkeit.

Verglichen mit der normalen Trächtigkeit waren die elektrischen Potentiale und elektrochemischen Gradienten beim Zustand der Hydrallantois ohne primäre Missbildungen der Frucht auffallend niedriger. Die Permeabilität der Allantochorion war bei einigen extremen Fällen der Krankheit in Vitro deutlich erhöht.

Die Ergebnisse lassen vermuten, dass die Pathogenese mit einem primär verringerten aktiven Ionentransport durch das Chorion gefolgt von einer mechanischen Zerstörung der Mikrostruktur des Allantochorions in Verbindung steht.

SAMMENDRAG

Patogenesen ved hydrallantois bovis.

II. Elektriske potentialer og kemiske gradienter mellem allantoisvæsken og det maternelle blod ved hydrallantois og normal drægtighed.

Sammenlignet med normal drægtighed fandtes de elektriske potentialer og elektrokemiske gradienter nedsat i tilfælde af hydrallantois uden primær misdannelse af fosteret. Allantochorions permeabilitet in vitro var tydeligt forøget i nogle extreme tilfælde af sygdommen.

Resultaterne lader formode, at patogenesen kan være forbundet med en primært nedsat aktiv iontransport gennem chorion efterfulgt af mekanisk destruktion af allantochorions mikrostruktur.

(Received December 17, 1964).